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GENERAL ARTICLE

Genetics and geography of leukocyte telomere length in sub-Saharan Africans

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Abstract

Leukocyte telomere length (LTL) might be causal in cardiovascular disease and major cancers. To elucidate the roles of genetics and geography in LTL variability across humans, we compared LTL measured in 1295 sub-Saharan Africans (SSAs) with 559 African–Americans (AAms) and 2464 European–Americans (EAms). LTL differed significantly across SSAs ($P = 0.003$), with the San from Botswana (with the oldest genomic ancestry) having the longest LTL and populations from Ethiopia having the shortest LTL. SSAs had significantly longer LTL than AAms [$P = 6.5$(e-16)] whose LTL was significantly longer than EAms [$P = 2.5$(e-7)]. Genetic variation in SSAs explained 52% of LTL variance versus 27% in AAms and 34% in EAms. Adjustment for genetic variation removed the LTL differences among SSAs. LTL genetic variation among SSAs, with the longest LTL in the San, supports the hypothesis that longer LTL was ancestral in humans. Identifying factors driving LTL variation in Africa may have important ramifications for LTL-associated diseases.

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Introduction

Telomere length (TL) undergoes age-dependent shortening in replicative somatic cells starting *in utero* (1). Epidemiological studies have used leukocyte TL (LTL) as a proxy of TL in other somatic tissues (2), and many researchers concluded that it is a passive biomarker of human aging and adult-onset diseases (3). Recent findings suggest, however, that LTL is a component in a causal pathway in such diseases. Several lines of evidence support this inference, including findings that LTL is largely determined at birth and in early childhood (1,4). Consequently, individuals who enter adulthood with comparatively long or short LTL typically have long or short TL throughout the rest of their lives (5). Thus, the risk of adult-onset diseases associated with long or short TL is often defined decades before overt manifestations of disease (6). In addition, genome-wide association studies have identified LTL-associated single-nucleotide polymorphisms (SNPs), the majority of which map to loci that harbor telomere maintenance genes (7–9). These loci have been used to infer, through Mendelian randomization analyses, a causal role of long telomeres in major cancers and short telomeres in atherosclerotic cardiovascular disease (10,11), the two disease categories that largely define longevity in middle- and high-income countries.

African–Americans (AAms) have longer LTL (12–16) and are less susceptible to atherosclerotic cardiovascular disease than individuals of European ancestry (16). In addition, a study of a small sample of Tanzanians, AAms and European–Americans (EAms) found longer LTL in Africans from Tanzania than in the other populations (14). The study also showed that the shifts in allele frequency between sub-Saharan Africans (SSAs) and individuals of European ancestry do not fit the pattern expected by neutral genetic drift of loci that affect LTL. This observation is consistent with the conclusion that TL is a target of selection, and that polygenic adaptation that contributes to differences in frequency of LTL-associated alleles in globally diverse populations can drive population variation in LTL (14). These findings demonstrate that to better understand adult-onset disease disparities across continental populations, we need to know more about the factors influencing LTL variation in diverse populations. The aim of this study was to estimate the diversity of and genetic contributions to LTL across a diverse set of sub-Saharan populations and test whether or not population differences in LTL can be explained by these genetic effects.

Results

LTL was measured by Southern blotting in 1295 SSAs from seven populations defined by geography (Botswana, Tanzania and Ethiopia) and genetically inferred ancestry (hereafter, referred to as 'ancestry') (17). These include hunter–gatherer populations (Khoisan-speaking San from Botswana and Hadza from Tanzania), pastoralists (Nilo-Saharan-speaking populations from Ethiopia and Herero from Botswana), agriculturalists (Bantu-speaking populations from Tanzania and Botswana) and agro-pastoralists (Afroasiatic-speaking populations from Ethiopia and Tanzania). Adults were randomly sampled from the different populations and lived in mostly rural areas practicing indigenous lifestyles. We compared LTL findings in these populations to 559 AAms and 2464 EAms from the Family Heart Study (12) with available genotypes for kinship estimation and LTL measurements for direct comparisons of LTL in SSA populations. AAms resided in Alabama and North Carolina, and EAms resided in Massachusetts, Minnesota, North Carolina and Utah.

We focused on whether the effects of genetics, age and sex on LTL explain the population differences in mean and variance of LTL. SSAs had significantly longer sex- and age-adjusted LTL than AAms [difference = 0.47 ± 0.06 kilobase pairs (kb), $P = 6.5(e\text{-}16)$], and AAms had significantly longer adjusted LTL than EAms [difference = 0.19 ± 0.04 kb, $P = 2.5(e\text{-}7)$] (Table 1). The sex- and age-adjusted LTL variances were significantly different among the three groups [Levene's test for homogeneity, $P = 2.2(e\text{-}14)$].

The San population in Botswana showed the longest age- and sex-adjusted LTL ($P < 0.001$ versus each of the other six SSA populations; $P = 0.003$ for the overall test of mean differences among all SSA populations; Table 1). The two ancestries in Ethiopia had the shortest adjusted LTL, and those with an Afroasiatic ancestry living in Ethiopia had shorter LTL than those living in Tanzania [difference = 0.26 ± 0.06 kb, $P = 2.7(e\text{-}6)$]. There was no significant difference in LTL between the Bantu-speaking populations in Botswana versus Tanzania (difference = 0.10 ± 0.08 kb, $P = 0.23$). These observations are consistent with a north–south gradient of increasing LTL.

Sex-adjusted LTL decreased with age at a rate of −0.023 ± 0.0010 kb/year in SSAs [$P = 1.8(e\text{-}114)$; Fig. 1]. The rate of decrease did not differ by SSA ancestral groups ($P = 0.15$). Within each ancestry group, all age regression coefficients with LTL were also significant. The age regression coefficient for sex-adjusted LTL in AAms was −0.024 ± 0.0022 [$P = 4.8(e\text{-}28)$] and in EAms was −0.022 ± 0.0009 [$P = 2.4(e\text{-}126)$]. The age regression coefficients were not significantly different from one another in a test for interaction across the three populations ($P = 0.61$).

SSA women had longer age-adjusted LTL than men, with a difference of 0.164 ± 0.039 kb [7.477 ± 0.054 kb in males and 7.642 ± 0.049 kb in females, $P = 2.2(e\text{-}05)$; individual population LTL differences by sex shown in Fig. 2]. For AAms and EAms, the LTL differences by sex were 0.266 ± 0.052 [$P = 2.7(e\text{-}7)$] and 0.159 ± 0.021 [$P = 1.7(e\text{-}14)$], respectively. There was no interaction between sex and ancestry in SSAs ($P = 0.64$), and there was no interaction of the sex differences in LTL among SSAs, AAms and EAms ($P = 0.16$).

The regression of LTL on age and sex explained 18% of the inter-individual variance of LTL in SSAs (Table 2). The total genetic variance of LTL, estimated using a linear mixed model with kinship coefficients derived from SNP data and after accounting for variation due to age and sex, represented an additional 52% of the total LTL variance [$P < 1(e\text{-}200)$]. Variation among the means of the seven SSA populations explained 10% additional variance of LTL [inter-ancestry genetic variance, $P = 7.2(e\text{-}23)$]; therefore, the remaining 42% was derived from intra-ancestral group genetic variance. Adjustment for the genetic variance of LTL in SSAs removed the significance of the mean LTL differences among the genetically inferred SSA ancestry groups ($P = 0.45$).

Table 2 shows that the percent of variation explained by age and sex was similar for SSAs, AAms and EAms (17–19%). The genetic variance of age- and sex-adjusted LTL in SSAs was almost twice as large as in AAms (52 versus 27%) and just over 50% greater than in EAms (52 versus 34%).

The effects of adjustment of LTL for genetic variance for each SSA ancestry group are shown in Figure 3. Adjustment for the genetic variance of LTL had the largest effect on the mean LTL of the San population in Botswana and made the LTL means across the seven ancestry groups non-significant ($P = 0.45$).

Table 1. Age- and sex-adjusted leukocyte telomere length means (±SEM) by country and ancestry

Population	LTL (kb)	Female (%)	Age (years)
European–Americans (*N* = 2464)	6.90 ± 0.02*	54.6	57.2 ± 13.3
African–Americans (*N* = 559)	7.09 ± 0.03*	66.2	53.3 ± 11.0
Sub-Saharan Africans (*N* = 1295)	7.56 ± 0.05*	52.6	39.4 ± 16.2
Ethiopia (*N* = 445)	7.38 ± 0.04	44.7	36.8 ± 14.3
Nilo-Saharan (*N* = 124)	7.35 ± 0.03	38.7	30.7 ± 11.3
Afroasiatic (*N* = 321)	7.41 ± 0.06	47.0	39.1 ± 14.6
Tanzania (*N* = 491)	7.59 ± 0.06	52.3	41.8 ± 17.5
Afroasiatic (*N* = 138)	7.67 ± 0.03	65.2	44.5 ± 15.5
Hadza (*N* = 246)	7.51 ± 0.05	48.0	40.8 ± 18.7
Bantu (*N* = 107)	7.45 ± 0.06	45.8	40.7 ± 16.8
Botswana (*N* = 359)	7.74 ± 0.07	62.7	39.5 ± 16.1
Bantu (*N* = 115)	7.55 ± 0.06	73.0	37.8 ± 15.0
San (*N* = 244)	7.89 ± 0.06†	57.8	40.3 ± 16.6

SEM, standard error of the mean; LTL, leukocyte telomere length.
LTL adjusted for sex, age and intra-population LTL correlations using generalized estimating equations.
*$P < 0.0001$ for each of the three pairwise comparisons of the European–American, African–American and sub-Saharan African populations.
†$P < 0.001$ for each comparison of LTL in San versus the other six sub-Saharan populations.

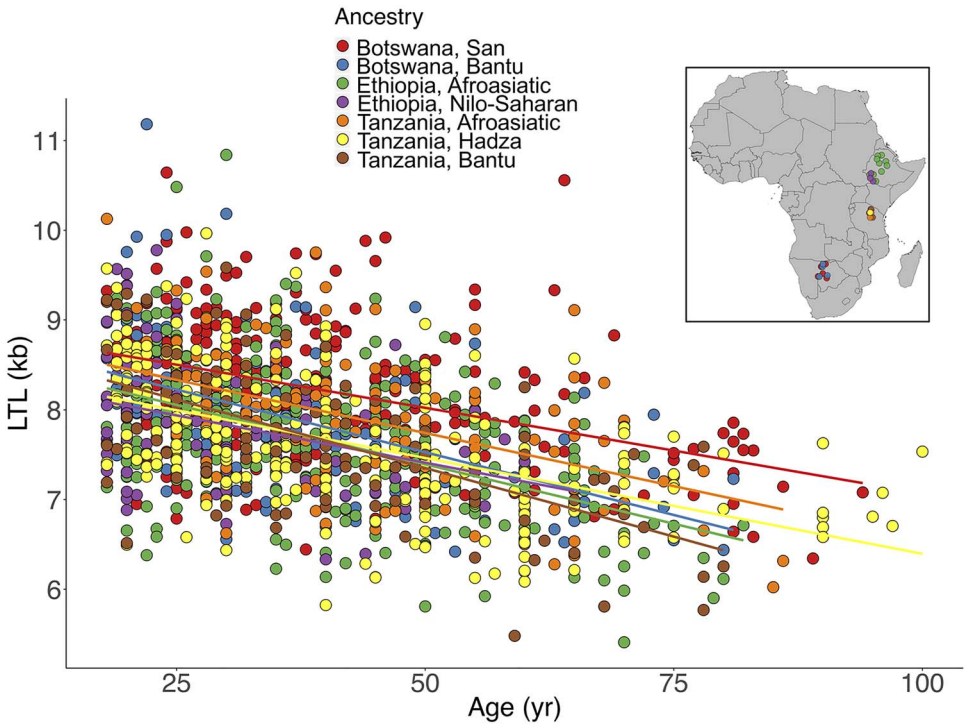

Figure 1. Association of leukocyte telomere length (LTL) with age for each ancestry (adjusted for sex). Linear regression lines of age versus LTL for each inferred ancestry are superimposed over the individual observations (*N* = 1295).

Table 2. Percent of leukocyte telomere length (LTL) variation (variance estimate in kb) explained by age, sex and genetics in the three study populations

Source of LTL variation	Sub-Saharan Africans	African–Americans	European–Americans
Age	17% (0.113)	15% (0.062)	15% (0.066)
Sex (age-adjusted)	1% (0.007)	4% (0.015)	2% (0.008)
Genetics (age- and sex-adjusted)	52% (0.342)	27% (0.110)	34% (0.153)
Full model (age, sex and genetics)	71% (0.462)	46% (0.187)	51% (0.227)
Total LTL variance	0.653	0.408	0.447

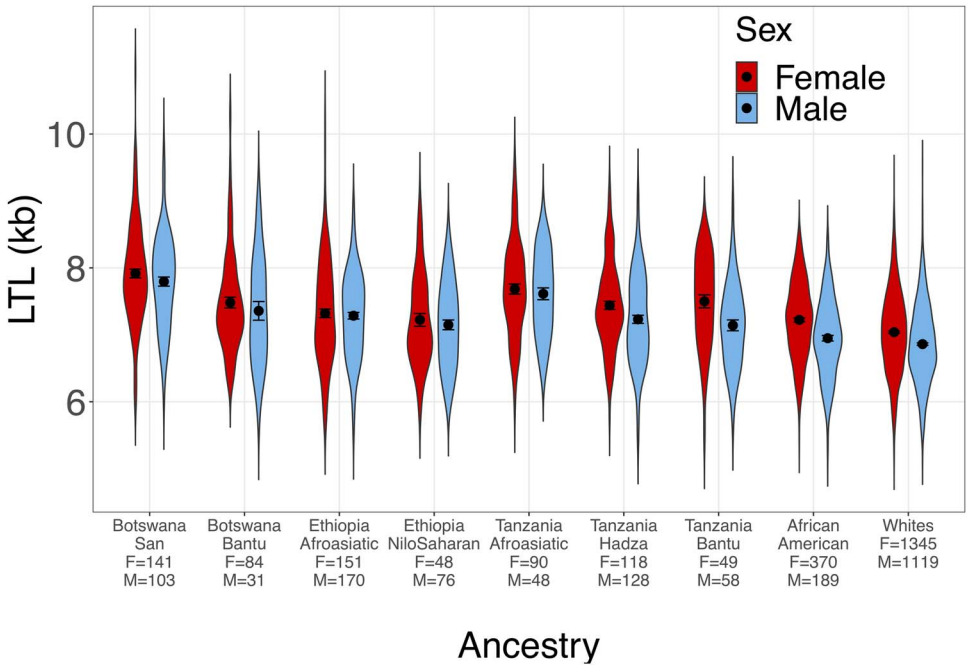

Figure 2. Sex differences in leukocyte telomere length (LTL) by ancestry (adjusted for age). Error bars are standard errors of the mean ($N = 1295$).

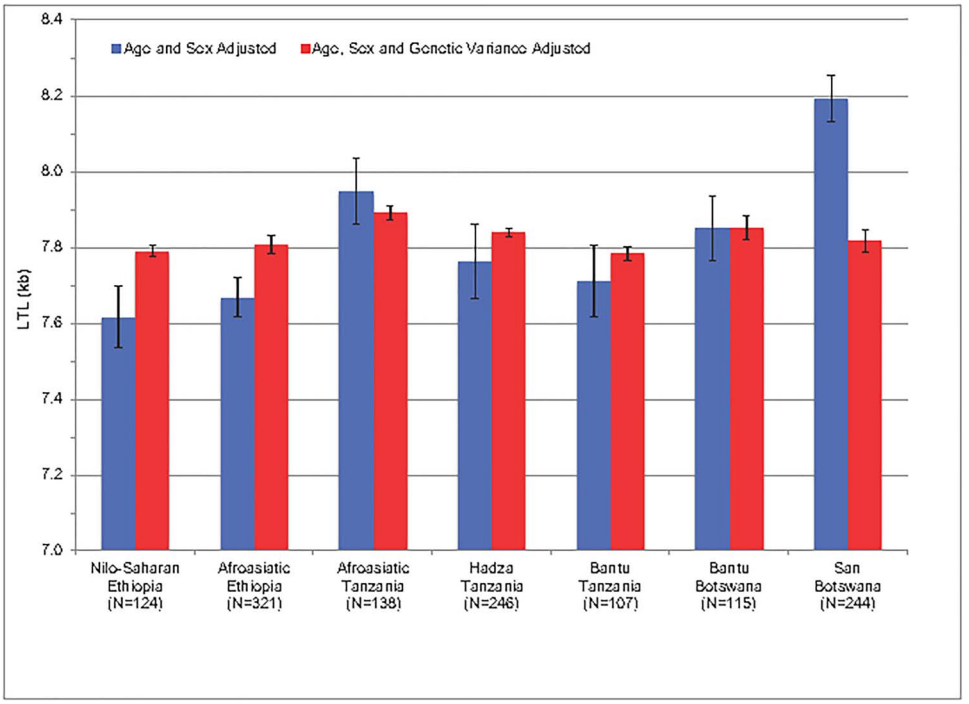

Figure 3. Leukocyte telomere length (LTL) by ancestry. LTL is shown adjusted for age and sex, and adjusted for age, sex and LTL genetic variance. The genetic variance–covariance structure of LTL was estimated using a kinship matrix calculated from identity-by-decent probabilities and using the estimated additive genetic variance, both derived using a genome-wide single-nucleotide polymorphisms array. Error bars are standard errors of the least square means ($N = 1295$).

Discussion

The key findings of this work are LTL in the San hunter–gatherers is longer than in other SSA populations, whose LTL is longer than in AAms and EAms (14). Coupled with findings of longer LTL in Hominidae (great apes) than humans (18,19), these findings are consistent with the hypothesis that LTL in ancestral humans was longer than in contemporary humans. Although SSAs have longer LTL than AAms and EAms (14), they share two important LTL-related features with non-Africans: a sex effect (4,5,20,21), which in non-Africans is already observed in newborns (no information is available at present on LTL in SSA newborns) (4), and a similar rate of age-dependent LTL shortening in adults (5,6,12), reflecting the systematic loss of telomere repeats with

replications of hematopoietic stem cells/progenitor cells (22,23). The consistency of the sex and age associations with LTL across adult populations living in different environmental conditions indicates that LTL differences among ancestry groups are largely independent of adult age and sex.

The total LTL variation and the genetic variance component of LTL estimated from SNP data in SSAs was larger than in the North American populations. The larger genetic variation may result from larger SNP effect sizes, altered allele frequencies or different genes responsible for LTL variation than in the other two populations. Importantly, adjustment for the genetic effects reduced the SSA residual LTL variance so that it was similar to the residual variance in AAms and EAms and removed the SSA population differences in mean LTL. Similarly, in the US Health and Retirement Study, the significant LTL difference between AAms and EAms was removed after adjusting for genetically derived principal components (15). The equalization of either the LTL variances or means in the two studies after adjustment for the genetic contribution to LTL suggests that genetics is the main determinant of variation in LTL among populations.

It is not clear why the SNP-estimated genetic variation of LTL in AAms is not intermediate between the SSAs and EAms. Despite the variation of the AAms being similar to EAms, it does not mean that the same variants or genes explain inter-individual LTL variation within and across the groups. In addition, most AAm ancestry is from western or south-western Africa, whereas the SSAs of this study are eastern and southern Africans. Other factors also influence LTL beyond age, sex and genetics, and these, operating in quite different environments in Africa and the USA, may contribute to the lower variance estimates observed in AAms.

In previous work we proposed that in the course of the north-bound migration out of Africa, Europeans experienced poly-genetic adaptation that contributed to the shortening of their LTL, perhaps attenuating the increased risk of individuals with de-pigmented skin to sporadic melanoma (14). Although this finding explains in part the shorter LTL in EAms, the precise evolutionary explanation for the persistence of long LTL in SSA populations is not understood. That said, birds with long telomeres display better survival when infected with avian malaria (24), and acute *Plasmodium falciparum* malaria infection increases the rate of LTL shortening in humans (25). Having long telomeres might thus be advantageous in surviving malaria and other viral and parasitic infections, further supporting the concept that LTL is the target of selection.

Finally, we acknowledge the following limitations of our study. First, the inclusion of newborns, children and individuals from populations in central and western Africa would provide a more comprehensive picture of LTL in SSAs. Second, measurements of mean LTL for this study and for the Family Heart Study were performed more than a decade apart, but were measured by an identical technique (26) performed in the same laboratory. Moreover, the same laboratory performed parallel LTL measurements and generated similar findings in samples from the Family Heart Study and from SSAs in a small study that provided the foundation for this work (14).

In conclusion, SSAs, who have the highest degree of genetic diversity (27), have on average a longer and more variable LTL than non-Africans. Optimal LTL in a population may be determined by pleiotropic telomere maintenance genes for a given, but as of yet unknown, environmental setting. Throughout human history, the characteristics and function of these genes may have been forged by past genetic/environmental forces that may not hold for modern populations. A possible outcome of LTL pleiotropy is that modern populations with long LTL experience higher rates of major cancers and populations with short LTL experience higher rates of atherosclerotic cardiovascular disease (10,11). Our findings call for intense research for the ramification of a longer LTL in SSAs in the context of their propensities for cancers and atherosclerotic cardiovascular disease. Insight into the genetic contributions that have maintained a longer LTL in SSAs will have, therefore, considerable ramifications from the standpoint of telomere-mediated cancer–atherosclerosis trade-off in humans (28).

Materials and Methods

Study design and participants

Mean LTL in SSAs was measured by Southern blotting of the terminal restriction fragments (26). All samples, measured in duplicate, passed a DNA integrity test before LTL measurements were obtained. The inter-assay intra-class correlation from duplicate measurements was 0.98 (0.91–1.0). We compared mean LTL findings in these populations to 559 AAms and 2464 EAms from the Family Heart Study (12). Identical LTL measurements were performed in the same laboratory for the present study and the Family Heart Study.

Adult participants were randomly recruited from each village. Written informed consent was obtained from all participants and research/ethics approval and permits were obtained from the following institutions prior to sample collection: The University of Pennsylvania, COSTECH, NIMR and Muhimbili University of Health and Allied Sciences in Dar es Salaam, Tanzania; the University of Botswana and the Ministry of Health in Gaborone, Botswana; the University of Addis Ababa and the Federal Democratic Republic of Ethiopia Ministry of Science and Technology National Health Research Ethics Review Committee.

Statistical analysis

The LTL distribution is normally distributed with very low skewness (0.27) and kurtosis (0.22). The variance in LTL due to genetic differences in LTL-related genes in the SSAs was estimated by first assigning ancestry based on genotypes rather than self-reported ancestry. Ancestry was inferred using unsupervised ADMIXTURE analysis (29) based on over 4.1 million SNP genotypes from a 5 M Omni Illumina array. Individuals were pooled based on shared ancestry at $K=5$ (five genetic ancestral populations), largely corresponding to linguistic classification [southern Khoesan (San), eastern Khoesan (Hadza), Afroasiatic, Bantu, Nilo-Saharan]. Individuals of Afroasiatic and Bantu ancestries resided in two different countries and were coded and analyzed after grouping by both ancestry and country (seven groups) to allow for migration or other geographical effects. EAm and AAm ancestries for subjects in the Family Heart Study were self-reported, but should be quite accurate based on a previous large study that included a subset of the Family Heart Study and validated self-reported ancestry (30). Genotypes were obtained from an Illumina HumMap550K, Human 6100-Quadv1 or Human 1 M-DuoV3 array for EAms or a Human 1 M-DuoV3 array for AAms.

For the estimation of age and sex associations with LTL within and across populations, regression using a general linear model (GLM) procedure was used (PROC GLM, SAS Inc., Cary, NC, USA). The combined sub-Saharan population age and sex associations and differences in mean LTL were also compared with

the AAm and EAm populations using GLM. Significance tests of the age and sex regression coefficients were obtained using generalized estimating equations (PROC GENMOD, SAS Inc.) and a compound symmetry variance–covariance matrix estimated within each population to account for non-independent observations due to relatedness. When presented separately, the sex effect was adjusted for age and the age effect was adjusted for sex. Interactions of sex and age with population were modeled by cross-products of the two variables, effectively testing for differing male and female LTL differences among populations or differing age versus LTL slopes among populations.

To adjust LTL for the total genetic variance, kinship coefficients were first estimated from identical-by-descent (IBD) estimates calculated from PLINK (31) for all pairs of individuals within each of the three population groups. Kinship was assumed to be zero between groups. Calculations in PLINK were based on the subset of SNPs in linkage equilibrium ($r^2 < 0.1$ for EAms and SSAs and $r^2 < 0.2$ for AAms) within windows of 100 kb. A different cutoff of linkage disequilibrium (LD) was used for AAms to include approximately the same number of SNPs within each window as for the other two populations for the IBD estimation. The genetic variance–covariance structure of LTL is estimated as $2 \times$ matrix of kinship coefficients $\times$ the additive genetic variance. The R polygenic_hglm function (32), which is part of the GenABEL package (33), was used to calculate LTL residuals from a model including age, sex and the kinship matrix estimated from pairwise IBD estimates from the study subjects. The difference between the variance of these residuals and the total unadjusted LTL variance is the total variance explained by the model (age, sex and genetics). The LTL variance explained by age and sex was similarly estimated prior to considering the genetic data. The significance of the polygenic effect was tested by comparing -2 times the difference in log-likelihood of the age- and sex-adjusted models with and without the polygenic effect.

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